Enhancing the Quantitative Capabilities of Surface Plasmon Resonance Spectroscopy

NIST scientists are developing technology to improve upon the current capabilities of the important proteomics tool – surface plasmon resonance (SPR). SPR is currently used for kinetics measurements of a binding partner to a known protein. The development of enhanced SPR capabilities for quantitative protein measurements and imaging will help enable next-generation clinical diagnostic and drug screening applications. This work will enable new innovations in the biopharmaceutical sector. NIST expects the work to enable improved quality control of antibody and protein production and capabilities for identifying functions of unknown proteins in on-line proteomics applications.

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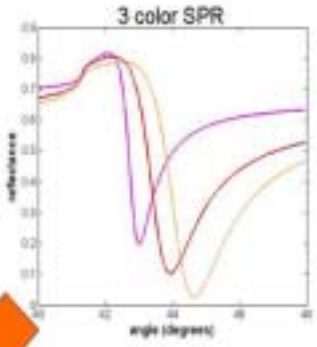
Surface plasmon resonance (SPR) spectroscopy is essentially a refractive index measurement that is sensitive to changes in mass at an interface with a metal substrate. This technique is ideal for measuring label-free protein interactions where a protein is immobilized to the sensor surface and ligand binding is measured from solution flow over the sensor.

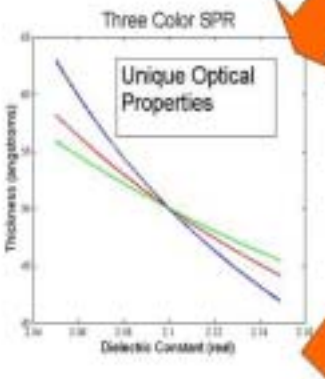
One of the primary functions of a protein is binding to a biomolecular partner. To measure this interaction, the quantity of free and bound protein fractions needs to be determined. SPR is ideal for this measurement because it does not require that the binding partners be labeled with external probes such as fluorophores or radiolabels to measure binding constants. Several commercial instruments are available and they are used widely in the biotechnology industries. One of the limitations of traditional SPR is that the measured response is a relative measurement with no absolute scale. Absolute number of proteins bound at an interface is the result of several experimental parameters such as density, protein saturation, on rate, off rate and the optical properties of the surface. Unambiguous characterization of the protein binding constants is not possible without additional measurements to remove the confounding variables.

NIST is developing new measurement and imaging tools based upon SPR. Enhanced SPR capabilities will include quantitative measurement of protein/receptor interactions, imaging and proteomics.

The major goals of the program are to: 1) make SPR more quantitative, 2) develop SPR as an imaging tool and 3) combine SPR with other techniques such as mass spectrometry (MS) to create a platform for capture and separation of unknown proteins.

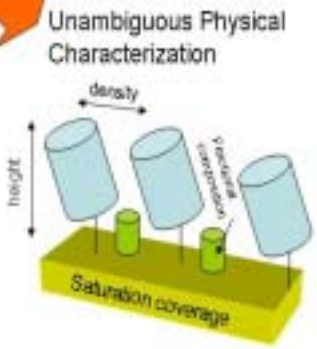
The first graph illustrates data collection methodology: collecting SPR reflectivity traces at 3 different wavelengths for one surface measurement.





The second graph illustrates the analysis technique for obtaining unique optical parameters: one wavelength reflectance trace provides a range of values to thickness vs. dielectric constant; 3 wavelengths provide an intersection and unique set of values for thickness and dielectric constant.

The third graphs illustrates that using unique values for thickness and dielectric constant one can interpret physical characteristics such as protein coverage unambiguously requiring no assumed parameters



We have designed a new multicolor SPR device and method of analysis to extract more information from each reflectance curve than is done with commercial instrumentation. Using multiple SPR scans at multiple wavelengths will allow for an unambiguous determination of the optical properties for the immobilized protein. A unique refractive index and optical thickness will allow for determination of protein coverage and allow for quantitative saturation measurements. This will allow

reliable measurements without assumptions including determining the effects of protein orientation on saturation binding, and determining equilibrium saturation directly instead of from association/dissociation kinetics.

Because SPR is an on-line measurement tool, it can be directly integrated into proteomic analysis instruments (i.e. mass spec) and allow measure of protein binding function with chemical characterization. SPR can be used for real-time immobilized ligand capture and as a separation technique whereby elution of the ligand off the surface is performed under a controlled method to separate specific ligand binding from nonspecific interactions.

Initial tests of sensitivity and analysis were performed on dodecanethiol and demonstrated expected results, confirming the system is performing as designed. A resolution of 10^{-5} degrees or 10^{-7} RIU (refractive index units) was obtained and for dodecanethiol, a literature value of 1.7nm thickness and dielectric constant of 2.15 (at 632nm) was confirmed.